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Mutagenicity in Salmonella of Hazardous Wastes and Urine from Rats Fed These Wastes

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MUTAGENICITY IN SALMONELLA OF HAZARDOUS WASTES AND URINE FROM RATS FED THESE WASTES

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ABSTRACT

Fifteen hazardous industrial waste samples were evaluated for mutagenicity in the Salmonella plate-incorporation assay using strains TA98 and TA100 in the presence and absence of Aroclor 1254-induced rat liver S9. Dichloromethane/methanol extracts of the crude wastes also were evaluated. Seven of the crude wastes were mutagenic, but only 2 of the extracts of these 7 wastes were mutagenic; extracts of 2 additional wastes also were mutagenic. In addition, 10 of the crude wastes were administered by gavage to F-344 rats, and 24-h urine samples were collected. Of the 10 raw urines evaluated, 3 were mutagenic in strain TA98 in the presence of S9 and β-glucuronidase. The 3 crude wastes that produced these 3 mutagenic urines were, themselves, mutagenic. Adequate volumes of 6 of the 10 raw urines were available for extraction/concentration. These 6 urines were incubated with β-glucuronidase and eluted through Sep-Pak® C_{18}/columns; the methanol eluates of 3 of the urines were mutagenic, and these were the same 3 whose raw urines also were mutagenic. In general, the C_{18}/methanol extraction procedure reduced the cytotoxicity and increased the mutagenic potency of the urines. To our knowledge, this is the first report of the mutagenicity of urine from rodents exposed to hazardous wastes. Based on the present results, the use of only strain TA98 in the presence of S9 might be adequate for general screening of hazardous wastes or waste extracts for genotoxicity. The urinary mutagenesis assay does not appear to be a useful adjunct to the Salmonella assay for screening hazardous wastes. The problems associated with chemically fractionating diverse types of hazardous wastes for bioassay are also discussed.

INTRODUCTION

The potential health effects of exposure to hazardous wastes may include chromosomal damage and cancer (Maugh, 1979; Vianna and Polan, 1984). Thus, knowledge of the mutagenic potential of hazardous wastes would aid greatly in evaluating the hazardous nature of and/or potential health effects from exposure to certain wastes. Most studies on the genotoxicity of hazardous wastes have used the Salmonella assay (Houk and Claxton, 1986; Nestmann et al., 1980); however, eukaryotic and/or mammalian cell assays have been used to a limited extent (DeMarini et al., 1984; Donnelly et al., 1985; Hopke et al., 1982). Combinations of short-term genotoxicity assays have been proposed as possible screens for hazardous wastes (Barfknecht and Naismith, 1984).

We have begun a series of studies to identify short-term assays and chemical fractionation schemes that might be useful for screening hazardous wastes for genotoxic activity. Our initial studies have indicated that the use of mammalian cell assays might not improve significantly the ability to detect the genotoxicity of hazardous wastes beyond that afforded by the Salmonella assay alone (DeMarini et al., 1987a). Because in vivo mammalian metabolism may be a critical factor in the generation of mutagenic metabolites from complex hazardous wastes, we have investigated the utility of a rodent urinary mutagenicity assay as a possible adjunct to testing the wastes themselves in the Salmonella assay.

Most previous studies with rodent urinary mutagenicity assays have involved the use of pure compounds--frequently potent mutagens known to require metabolic activation (Legator et al., 1982). Consequently, the raw urine from such studies was mutagenic and did not require concentration. However, β-glucuronidase was usually required to observe rodent urinary mutagenicity

in these studies. This is in contrast to studies of mutagenic activity of human urine in which the urines must be concentrated (usually with XAD resins), and β-glucuronidase is usually not required (Legator et al., 1982).

We are aware of only 3 studies on the mutagenicity of urine from rodents exposed to complex mixtures. Belisario et al. (1984, 1985) demonstrated the mutagenicity of urine from rats exposed by gavage to diesel emission particles (DEP), whereas Ong et al. (1985) did not find mutagenic urine from rats exposed by inhalation to DEP and/or coal dust. Concentration of the urine by XAD-2/acetone did not reveal any mutagenicity in one study (Ong et al., 1985), and it reduced the mutagenic potency compared to raw urine in the other studies (Belisario et al., 1984, 1985). Solid-phase extraction using Sephadex LH-20 resin or liquid extractions using chloroform, toluene, or dichloromethane (DCM) also reduced the mutagenic potency compared to raw urine (Belisario et al., 1985).

However, Ball et al. (1984) found that the mutagenic activity of urine from rats administered 1-nitropyrene was enhanced by extracting and concentrating the urine on Sep-Pak® C_{18} cartridges eluted with methanol. Consequently, we have studied the mutagenicity of urine from rats exposed by gavage to crude hazardous wastes by testing both the raw urine as well as C_{18}/methanol concentrates of the urine in strain TA98 in the presence of Aroclor 1254-induced rat liver S9 and β-glucuronidase.

Previous studies have shown that DCM may extract mutagenic organics from hazardous wastes (Andon et al., 1985; DeMarini et al., 1987a). Thus, we have explored the applicability and utility of preparing and bioassaying DCM (as well as methanol) extracts of the 15 hazardous waste samples used in the present study. We selected samples that were representative of wastes from diverse manufacturing plants, including petrochemical, pharmaceutical, and plastics manufacturing plants. We also tested combinations of wastes from a variety of

sources. Some of the samples were aqueous wastes, some were organic wastes, and others were a mixture of both types. Some wastes were from a single manufacturing process; others were composite wastes from a variety of different manufacturing processes. All of the wastes were liquid wastes, although many contained solids. The fact that these 15 waste samples had been partially chemically characterized (U.S. EPA, 1984), which is information not normally available for hazardous wastes or other complex mixtures, provided an additional reason for using these wastes to explore bioassay procedures appropriate for hazardous wastes.

MATERIALS AND METHODS

Wastes and Waste Extracts

Fifteen samples of hazardous industrial wastes were obtained from Edward L. Katz, Hazardous Waste Engineering Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH (Table 1). Three of the waste samples (A, B, and C) were from 3 different manufacturing plants (petrochemical, pharmaceutical, and plastics). The remaining samples were from 4 commercial hazardous waste incineration facilities that burn a mixture of hazardous wastes from a variety of industrial sources.

Table 1 shows the results of a partial chemical characterization that was performed on these samples (U.S. EPA, 1984). For the purposes of a previous study on the performance of hazardous waste incinerators (U.S. EPA, 1984), 8 of the waste samples (B, E, F, G, J, K, L, and M) were spiked with carbon tetrachloride and trichloroethylene, which are not mutagenic in Salmonella (Kier et al., 1986). Consequently, the concentrations of these 2 chemicals in these 8 waste samples reflect this addition (Table 1). The wastes were analyzed for the presence of a limited number of priority organics and/or metals identified in the EPA Appendix VIII list of priority pollutants (U.S. EPA, 1984). Thus, the chemical characterizations shown in Table 1 should not be viewed as indicative of the overall chemical composition of the wastes. The procedures used to extract the wastes and to evauate the wastes and waste extracts in the Salmonella plate-incorporation assay have been described previously (DeMarini et al., 1987b).

Urine and Urine Extracts

Ten of the 15 waste samples were evaluated for their ability to produce mutagenic urine based on the chemical diversity that they represented. We had planned to dose animals with 4 different doses for 10 days before collecting

24-h urine from 3 rats per dose. However, the available amounts of waste samples permitted the use of this protocol for only waste C. For the other 9 waste samples, a single dose of the crude waste samples was administered by gavage to 70-day-old male F-344 rats. Doses were selected based on lethality and the maximum volume that could be administered to the animals. After dosing, rats were placed in metabolic cages (Nalgene), and urines were collected on dry ice for 24 h from 2-3 rats receiving the same dose of a particular waste. All of the urines were centrifuged (10,000 rpm, 10 min.), filter sterilized (0.45-μm filter), and frozen at -20°C.

Portions of some of the urines were processed further as follows. One ml of β-glucuronidase (Sigma Type VII from Escherichia coli) at a concentration of 1000 units/ml of potassium phosphate buffer (0.15 M, pH 7.4) was added to 2-10 ml of thawed urine, and the mixtures were incubated with shaking for 1 h at 37°C. Two serially connected Sep-Pak® C_{18} cartridges (Waters Associates, Milford, MA) containing 1 g of adsorbent were prepared for use by eluting 40 ml of methanol followed by 50 ml of double-distilled water. Then, each β-glucuronidase-urine incubate was poured through the columns, followed by 40 ml of double-distilled water. Water was aspirated from the columns until dry. Concentrates were then eluted with 2, 4-ml aliquots of methanol. The methanol concentrates were evaporated under a stream of nitrogen and solvent exchanged into a volume of DMSO to produce 5-X concentrates. These were stored at -20°C until they were bioassayed. Thus, there were two types of urine for bioassay: (1) raw urine and (2) urine that had been incubated with β-glucuronidase and extracted and concentrated 5X by means of C_{18}/methanol elution. The Salmonella plate-incorporation assay was performed as described (Maron and Ames, 1983; DeMarini et al., 1987b).

RESULTS

Of the 15 crude wastes evaluated for mutagenic activity in Salmonella, 7 were mutagenic (Table 3). The mutagenic potencies (rev/µg) ranged from 1 for waste G and O, to over 1000 for waste A. Four of the wastes (L, M, G, and O) were positive only in strain TA98, and all but waste G required S9. Three wastes (C, A, and F) were positive only in strain TA100, and they were positive in the presence and absence of S9. Dose-response curves for the mutagenic wastes are shown in Fig. 1 (circles).

Extracts (Table 2) were prepared for all of the wastes except for waste B, which reacted violently with methanol--leading to the loss of the sample. Of the 14 waste extracts evaluated, 4 were mutagenic, and their mutagenic potencies ranged from 2 for extract H, to over 5000 for extract C (Table 3). Two extracts (H and J) were mutagenic only in strain TA98; extract A was mutagenic only in strain TA100; and extract C was mutagenic in both strains. Two extracts (C and J) required S9, and 2 (H and A) were positive in the presence and absence of S9. Dose-response curves for the mutagenic extracts are shown in Fig. 1 (squares).

Raw urine from untreated control rats (up to 1 ml per plate) was not mutagenic in strain TA98 plus S9 plus β-glucuronidase (Fig. 2). Of the 10 wastes evaluated for urinary mutagenicity, 3 (C, L, and M) were mutagenic (Table 4). Sufficient volumes of urine were available for C_{18}/methanol concentration for only 6 of the 10 urines studied. The concentration procedure did not reveal mutagenic activity that was not already evident from raw urine for these 6 urines. Thus, the 3 mutagenic raw urines (C, L, and M) were also the only mutagenic urine extracts (Table 4).

The C_{18}/methanol concentration procedure appeared to eliminate cytotoxic components from the urines and to enhance the mutagenic potencies of the

mutagenic urines (Table 4, Fig. 2). In addition to dose responses based on the amount of urine per plate, we used waste C, which had been administered for 10 consecutive days, to generate dose responses based on the amount of waste/rat (Fig. 2). A qualitative summary of all of the data is shown in Table 5.

DISCUSSION

Mutagenicity of Urine and Urine Extracts

Based on the wastes used here, the urinary mutagenesis assay does not appear to provide any additional useful information beyond that obtained from testing the wastes or waste extracts directly in the Salmonella assay. Only 3 of the 10 wastes tested produced mutagenic raw urine (Table 4), and all 3 of these wastes were mutagenic when tested directly in Salmonella (Table 3). Combined with the additional expense and time required to perform the urinary mutagenesis assay, this assay does not appear to be a useful adjunct to testing the wastes or waste extracts directly for mutagenicity.

Although the urinary mutagenesis assay appears to be of limited usefulness in screening hazardous wastes, the data presented here are, to our knowledge, the first report of the mutagenicity of urine from rodents fed hazardous wastes. The results raise the issue of whether the mutagenic component(s) of the hazardous wastes were excreted unmetabolized or were converted to mutagenic metabolites. Table 3 shows that, in general, the 3 wastes that produced mutagenic urine (wastes C, L, and M or their extracts) required S9 in order to be mutagenic in strain TA98. Our preliminary studies (data not shown) indicate that β-glucuronidase was required to detect mutagenic activity in the urine or urine extracts. This is consistent with findings for many pure compounds studied in rodent urinary mutagenesis assays (Legator et al., 1982) and suggests that certain components of the wastes were metabolized and conjugated to glucuronide.

Fractionating and concentrating the urines by means of the C_{18}/methanol procedure did not identify a urine to be mutagenic that was not identified as mutagenic from the studies with raw urine (Table 4). Thus, this additional procedure did not enhance overall detection capabilities. However, the C_{18}/methanol concentration procedure did appear to enhance the mutagenic potency of the

three mutagenic urines (Table 4, Fig. 2). The procedure either concentrated mutagenic components of the urine and/or reduced the concentration of cytotoxic components in the urines, permitting better detection of the mutagenic activity. The procedure did not increase to the same extent the mutagenic potency of the three urines. As shown in Fig. 2, it enhanced considerably the detection of the mutagenicity of urine from rats fed waste L, but it did not increase the potency of the urine from rats fed waste C (at least up to 0.5 ml or ml equivalents/plate).

Mutagenicity of Wastes and Waste Extracts

The additional time and expense required to prepare the extracts did not produce extracts that yielded much additional information that was not obtainable from the crude wastes. Table 3 shows that 7 crude wastes were mutagenic, but for only 2 of these (A and C) were the extracts mutagenic. However, 2 wastes (H and J) were identified as mutagenic based solely on the mutagenicity of their extracts. Thus, out of a total of 9 mutagenic wastes, only 2 (22%) would have been missed if only the crude wastes had been tested. It is important to note that not all wastes can be tested directly due to microbial contamination or physical state, e.g., viscosity, pH, etc. Thus, extraction/fractionation procedures will be necessary for some, if not most, hazardous wastes in order to examine their biological activity.

Design of Testing Schemes

Consideration also must be given to how complex a testing matrix needs to be in order to have an acceptable detection capability and still be cost-effective. For the 15 wastes tested, the matrix shown in Table 3 requires the production of 120 dose-response curves, each with at least 3 or 4 points, each in duplicate, and each repeated. (Inclusion of the urine assay for all 15 wastes would add

an additional 30 dose-response curves.) Although only 2 strains and 2 activation conditions were used, would a reduced test matrix have produced an acceptable level of detection for these waste samples?

Judicious selection of a test matrix (and test battery) are required to screen hazardous wastes for biological activity in a cost-effective manner without an unacceptable loss of detection capability. Considering the effort required to produce a test matrix as large as the one in Table 3, the use of only strain TA98 + S9 would have reduced the time and expense considerably, with the concomitant loss of only ~20% of the detection capability provided by the test matrix in Table 3. Consistent with this proposal is the finding by the National Toxicology Program that the use of strain TA98 or TA100 in the presence of S9 detects approximately 80% of the mutagenic pure compounds in their data base that were identified using 4 strains with and without S9 (Zeiger et al., 1985).

Chemical Analysis and Biological Activity

Although extremely limited, the partial chemical characterization of the waste samples (Table 1) is more extensive than would be available for most waste samples--the vast majority of which would be of completely unknown chemical composition. Because of the limitations of the chemical analysis, a comprehensive picture of the composition of each waste sample is lacking. Consequently, the chemistry does not provide a ready explanation of the biological effects. For example, a comparison of the mutagenicity of the aqueous to the organic phase of the waste samples shows that the organic phase is more mutagenic than the aqueous phase for 3 out of 4 sets of waste samples (Table 3). The wastes were analyzed for the presence of only a few chemicals, representing a few chemical classes (organic solvents, chlorinated organics, and metals). However, few of these agents are mutagenic in Salmonella (Kier et al., 1986); thus, the compounds

and their concentrations shown in Table 1 do not predict or even suggest the mutagenic activity exhibited by some of the wastes.

This raises the issue of the relevance of this type of chemical analysis, which is expensive and time-consuming to obtain, for predicting the potential mutagenicity (or other biological activities) of hazardous wastes. In order to explore this problem further, Simmons et al. (1987) have examined the lethality and hepatic toxicity of 10 of these waste samples in the rat. The presence of known hepatotoxins in these wastes suggested that some of the wastes might be hepatotoxic. However, the chemistry did not predict the lethality of the waste samples.

In our search for a suitable short-term test to either complement or replace the Salmonella assay for screening hazardous wastes, we also have examined the ability of these wastes to induce bacteriophage lambda (Houk and DeMarini, 1987). The results indicated that this assay was more sensitive than the Salmonella assay, and it was comparable to the Salmonella assay in terms of time and expense.

Current schemes used by the U.S. EPA and other agencies to identify wastes as hazardous rely primarily on physical characteristics and chemical composition of the wastes (Friedman, 1985). Greer (1984) has discussed the limitations of the current definition of hazardous waste, and the U.S. EPA has published possible guidelines to correct this situation by adding health effects, including mutagenicity data, to the evaluation of hazardous wastes (Federal Register, 1983; 1984a,b).

Both government (Federal Register, 1983; 1984a,b) and industry (Barfknecht and Naismith, 1984; Guiney, 1985) have recognized the important role that short-term tests could play in the toxicological assessment of hazardous wastes. This concensus has prompted us to investigate the feasibility of such a proposal.

Based on our present results, short-term bioassays may offer an important insight into the potential health effects of wastes and, thus, may aid in the identification of some wastes as hazardous.

REFERENCES

Andon, B., M. Jackson, V. Houk and L. Claxton (1985) The evaluation of chemical and biological methods for the identification of mutagenic an cytotoxic waste samples, in: J.K. Petros, W.J. Lacy and R.A. Conway (Eds.) Hazardous Industrial Solid Waste Testing, ASTMP Publ. Code No. 04-886000-16, Philadelphia, pp. 204-215.

Ball, L.M., M.J. Kohan, J.P. Inmon, L.D. Claxton and J. Lewtas (1984) Metabolism of 1-nitro[^{14}C]pyrene *in vivo* in the rat and mutagenicity of urinary metabolites, Carcinogenesis, 5, 1557-1564.

Barfknecht, T.R., and R.W. Naismith (1984) Methodology for evaluating the genotoxicity of hazardous environmental samples, Hazard. Waste, 1, 93-109.

Belisario, M.A., V. Buonocore, E. De Marinis and F. De Lorenzo (1984) Biological availability of mutagenic compounds adsorbed onto diesel exhaust particulate, Mutation Res., 135, 1-9.

Belisario, M.A., C. Farina and V. Buonocore (1985) Evaluation of concentration procedures of mutagenic metabolites from urine of diesel particulate-treated rats, Toxicol. Lett., 25, 81-88.

DeMarini, D.M., P.A. Brimer and A.W. Hsie (1984) Cytotoxicity and mutagenicity of coal oils in the CHO/HGPRT assay, Environ. Mutagen., 6, 517-527.

DeMarini, D.M., D.J. Brusick and J. Lewtas (1987a) Use of limited protocols to evaluate the genotoxicity of hazardous wastes in mammalian cell assays: Comparison to Salmonella, J. Toxicol. Environ. Health (in press).

DeMarini, D.M., J.P. Inmon, J.E. Simmons, E. Berman, T.C. Pasley, S.H. Warren and R.W. Williams (1987b) Mutagenicity in Salmonella of hazardous wastes and urine from rats fed these wastes, Mutat. Res. (in press).

Donnelly, K.C., K.W. Brown, J.C. Thomas and P. Davol (1985) Evaluation of the hazardous characteristics of two petroleum wastes, Hazard. Waste Hazard. Mater., 2, 191-208.

Federal Register (1983) Notification requirements; reportable quantity adjustments, Vol. 48, No. 102, May 25, pp. 23552-23602.

Federal Register (1984a) Hazardous waste management systems, Vol. 49, No. 32, February 15, pp. 5854-5859.

Federal Register (1984b) Proposed deadlines for exposure assessment, Vol. 49, No. 227, November 23, pp. 46304-46312.

Friedman, D. (1985) An overview of selected EPA RCRA test method development and evaluation activities, in: J.K. Petros, W.J. Lacy and R.A. Conway (Eds.) Hazardous Industrial Solid Waste Testing, ASTMP Publ. Code No. 04-886000-16, Philadelphia, pp. 77-84.

Greer, L.E. (1984) Definition of hazardous waste, Hazard. Waste, 1, 309-322.

Guiney, P.D. (1985) Use of predictive toxicology methods to estimate relative risk of complex chemical waste mixtures, Hazard. Waste Hazard. Mater., 2, 177-189.

Hopke, P.K., M.J. Plewa, J.B. Johnston, D. Weaver, S.G. Wood, R.A. Larson and T. Hinesly (1982) Multitechnique screening of Chicago municipal sewage sludge for mutagenic activity, Environ. Sci. Technol., 16, 140-147.

Houk, V.S., and L.D. Claxton (1986) Screening complex hazardous wastes for mutagenic activity using a modified version of the TLC/Salmonella assay, Mutation Res., 169, 81-92.

Houk, V.S., and D.M. DeMarini (1987) Use of the Microscreen phage-induction assay to assess the genotoxicity of 14 hazardous industrial wastes, (submitted).

Kier, L.D., D.J. Brusick, A.E. Auletta, E.S. Von Halle, M.M. Brown, V.F. Simmon, V. Dunkel, J. McCann, K. Mortelmans, M. Prival, T.K. Rao, and V. Ray (1986) The Salmonella typhimurium/mammalian microsomal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutation Res., 168, 69-240.

Legator, M.S., E. Bueding, R. Batzinger, T.H. Connor, E. Eisenstadt, M.G. Farrow, G. Ficsor, A. Hsie, J. Seed and R.S. Stafford (1982) An evaluation of the host-mediated assay and body fluid analysis, Mutation Res., 98, 319-374.

Maron, D.M., and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutation Res., 113, 173-215.

Maugh, T.H. (1979) Toxic waste disposal a growing problem, Science, 204, 819-823.

Nestmann, E.R., E.G.H. Lee, T.I. Matula, G.R. Douglas and J.C. Mueller (1980) Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay, Mutation Res., 79, 203-212.

Ong, T., W.-Z. Whong, J. Xu, B. Burchell, F.H.Y. Green and T. Lewis (1985) Genotoxicity studies of rodents exposed to coal dust and diesel emission particulates, Environ. Res., 37, 399-409.

Simmons, J.E., D.M. DeMarini and E. Berman (1987) Hepatotoxic and lethal effects of hazardous industrial wastes (submitted).

U.S. EPA (1984) Performance Evaluation of Full-Scale Incinerators, National Technical Information Center Publ. No. PB85-129500.

Vianna, N.J., and A.K. Polan (1984) Incidence of low birth weight among Love Canal residents, Science, 226, 1217-1219.

Zeiger, E., K.J. Risko and B.H. Margolin (1985) Strategies to reduce the cost of mutagenicity screening with the Salmonella assay, Environ. Mutagen., 7, 901-911.

TABLE 1

Concentration of Chemicals and Metals Identified in Hazardous Wastes (μg/g)

Chemical/Metal[a]	Petro-Chemical	Pharma-ceutical	Plastics	Hazardous Waste Incineration Facilities									
				1		2				3		4	
				Aqueous	Organic	Organic		Aqueous		Organic		Organic	Aqueous
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Aniline		14000	550000												
Benzyl Chloride	3000														
Bis-(2-ethyl-hexyl)-phthalate		500		< 100	3800							200	230	< 10	< 10
Butylbenzyl-phthalate				< 100	320	120	450	< 5	8	160	140				
Chlordane												< 60	< 60	19000	19000
Chlorophenylisocyanate		21000													
Cresol(s)										2000	2500				
Diethylphthalate						620	1300	240	240						
m-Dichlorobenzene		23000													
o-Dichlorobenzene		46000													
p-Dichlorobenzene		59000													
m-Dinitrobenzene			< 100												
Diphenylamine			6200												
2,4-Dimethylphenol										500	2000				
Hexachlorobutadiene												< 10	< 10	< 10	< 10
Hexachloroethane	560														
Hexachlorocyclo-pentadiene												< 10	< 10	230	260
Isophorone				< 100	110										
Mononitrobenzene			< 100												
Naphthalene	< 100			< 100	350	250	450	44	49	450	490	38	33	< 10	< 10
Phenol				34000	1000	1500	1700	1800	2900						
Phenylenediamine			2300												
Phenylisocyanate		160000													
Trans-1,4-dichloro-2-butene	59000														
1,2,4-Trichlorobenzene		290													
Benzene				< 3	260							46000	58000	< 10	< 10
Carbon tetrachloride	68000	44000		< 2	6000	3700	4400	< 10	< 10	5900	5700	9100	11300	< 10	< 10
Chlorobenzene		4100										390	500	< 10	< 10
Chloromethane		1200													
Chloroform	2900					170	270	21	22			110	60	< 10	< 10
Cis-1,4-dichloro-2-butene	18000														
Methylene Bromide												< 10	< 10	3100	4700
Methylene Chloride	21000											100	340	44	60
Methyl Ethyl Ketone				27	9700					18000	38000				
Tetrachloroethylene	11000			< 1	28	7100	9800	1600	1300	7900	8100	87	150	< 10	< 10
Toluene	240000			110	2400	32000	45300	2900	2700	56000	48000	160000	390000	22	20
1,1,1-Trichloroethane	< 100									24000	16000	330	230	< 10	< 10
Trichloroethylene	4000	40000		< 1	5500	3700	4400	90	85	8100	7800	8300	10300	< 10	< 10
Ag	< 3					< 1	< 1	< 1	< 1			< 1	< 1		
As	< 24					< 20	< 20	< 20	< 20	< 14	< 14	< 23	< 23		
Ba	< 7					110	140	6	7	1160	1150	990	1100		
Be	< 2					< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1		
Cd	< 5					6	6	< 1	< 1	153	15	49	55		
Cr	< 5					50	57	3	3	431	425	250	290		
Hg	< 22					< 10	< 10	< 10	< 10	< 4	< 4	< 50	< 50		
Ni	< 68					7	7	2	2	26	27	< 4	< 4		
Pb	< 19					140	150	< 10	11	1830	1800	1200	1300		
Sb	< 12					58	61	< 10	< 10	437	373	< 24	< 24		
Se	< 470					340	< 100	< 100	< 100	< 21	< 21	< 160	< 160		
Tl	< 23					< 20	< 20	< 20	< 20	< 9	< 9	< 22	< 22		
Si										< 1	< 1				
pH		2		8		7	7	6	7				7	7	7
Water (%)	2		5	94	3	38	48	95	67	21	23	5	5	94	96

[a] Data from EPA (1984). Waste samples B, E, F, G, J, K, L, and M were spiked with carbon tetrachloride and trichloroethylene; therefore, the concentrations of these 2 chemicals in these wastes reflect this addition.

TABLE 2

SOLVENT EXTRACTIONS OF HAZARDOUS WASTES

Waste	Phase separation[a] DCM	Methanol	Final volume[b] (ml)
A	No	No	4
B	No	--[c]	
C	No	No	5
D	Yes		2
E	No	No	2
F	No	Yes	2.5
G	Yes		2.5
H	No[d]	Yes	2
I	Yes		2
J	No	No	5
K	No	Yes	2
L	No	No	7
M	No	No	5
N	Yes		2
O	Yes		2

[a]Ten ml of each waste was subjected to extraction as described in Materials and Methods.

[b]Final volumes vary because variable amounts of DMSO were required to reconstitute each sample due to the different solubilities of the samples.

[c]Explosive reaction occurred upon the addition of methanol; no extract was available for bioassay.

[d]Phase separation occurred when both solvents were added; DCM phase was used for bioassay.

TABLE 3

MUTAGENIC POTENCIES OF WASTES AND WASTE EXTRACTS IN SALMONELLA

	Revertants per µg[a]							
	Crude Wastes				Waste Extracts			
	TA98		TA100		TA98		TA100	
Waste	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
A			1870	1326			79	37
B					NT[b]	NT	NT	NT
C			18	329	5581		168	
F			3	4				
G	1	1						
H					6	2		
J					3177			
L	58							
M	31							
O	1							

[a]Values are the model slopes calculated from the dose-response curves as described in the Materials and Methods. Blank areas represent nonmutagenic responses. Wastes or extracts of samples D, E, I, K, and N were not mutagenic. The average DMSO control values (rev/plate ± S.D.) were: 52 ± 8 (TA98 + S9), 33 ± 5 (TA98 - S9), 156 ± 19 (TA100 + S9), 151 ± 12 (TA100 - S9).

[b]NT, not tested.

TABLE 4

MUTAGENIC POTENCIES OF URINES AND URINE EXTRACTS IN STRAIN TA98

Waste	Amount of waste per rat (g/kg)	Revertants per ml or ml equivalents[a]	
		Raw	extracts
C	0.2	395	455
L	2.5	259	1586
M	5.0	63	205
G	5.0		
O	5.0		
E	5.0		
H	5.0		NT
J	2.5		NT
K	2.5		NT
B	5.0		NT

[a]Values were calculated from the linear portion of the dose-response curves as described in the Materials and Methods. Urines were tested in the presence of S9 and β-glucuronidase. Blank areas represent nonmutagenic responses. The average control (no urine) value ± S.D. was 41 ± 6.

TABLE 5

SUMMARY OF RESPONSES

Waste	Wastes[a]		Urines	
	Crude	extracts	Raw	extracts
C	+	+	+	+
L	+	-	+	+
M	+	-	+	+
G	+	-	-	-
O	+	-	-	-
E	-	-	-	-
H	-	+	-	NT
J	-	+	-	NT
K	-	-	-	NT
B	-	NT[b]	-	NT
A	+	+	NT	NT
F	+	-	NT	NT
D	-	-	NT	NT
I	-	-	NT	NT
N	-	-	NT	NT

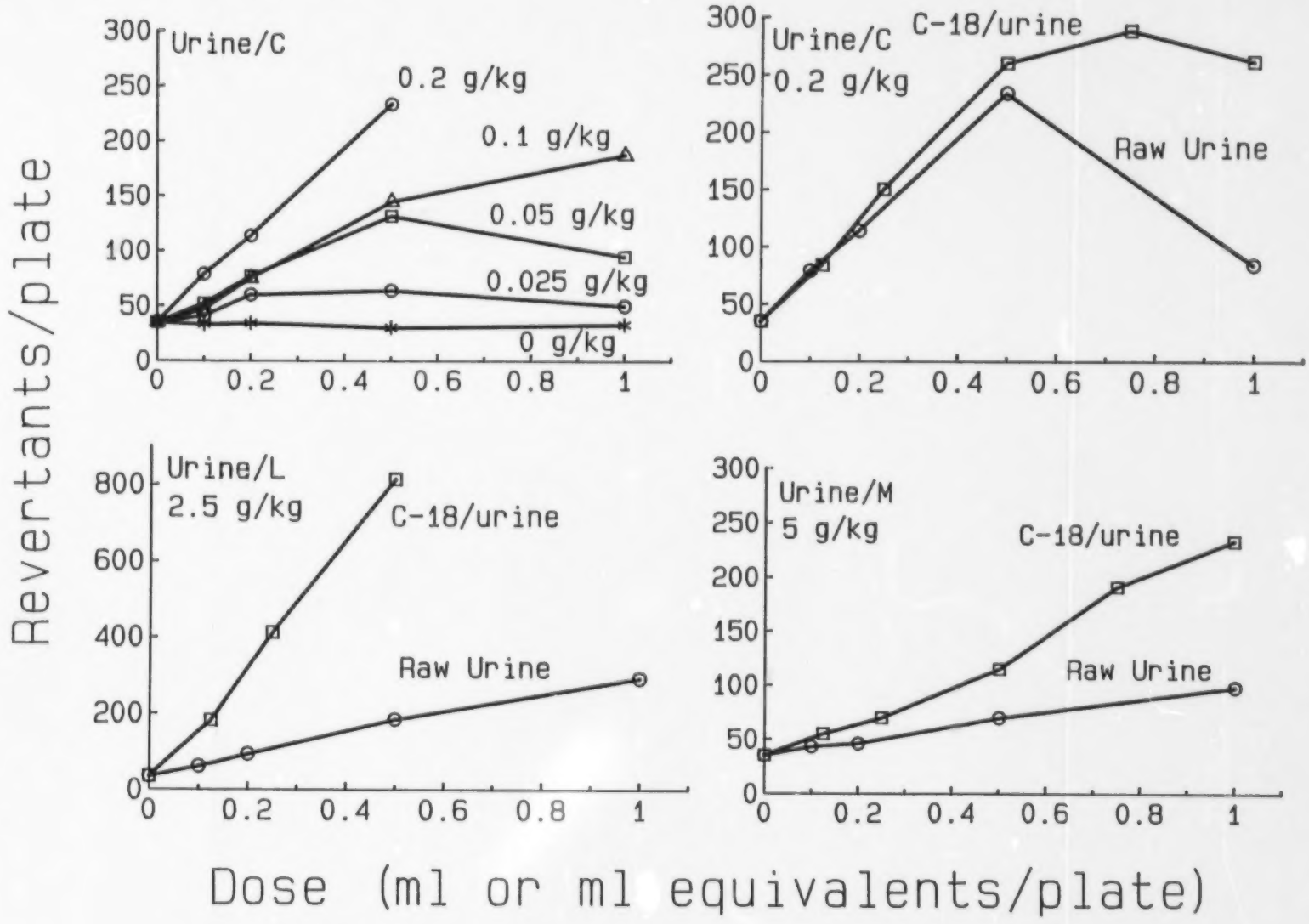
[a]Four responses (2 strains +/- S9) were merged into one response for both the crude wastes and the waste extracts (a total of 8 responses) using the following criterion: If a positive response was obtained under at least 1 of the 4 test conditions, the summary response was positive.

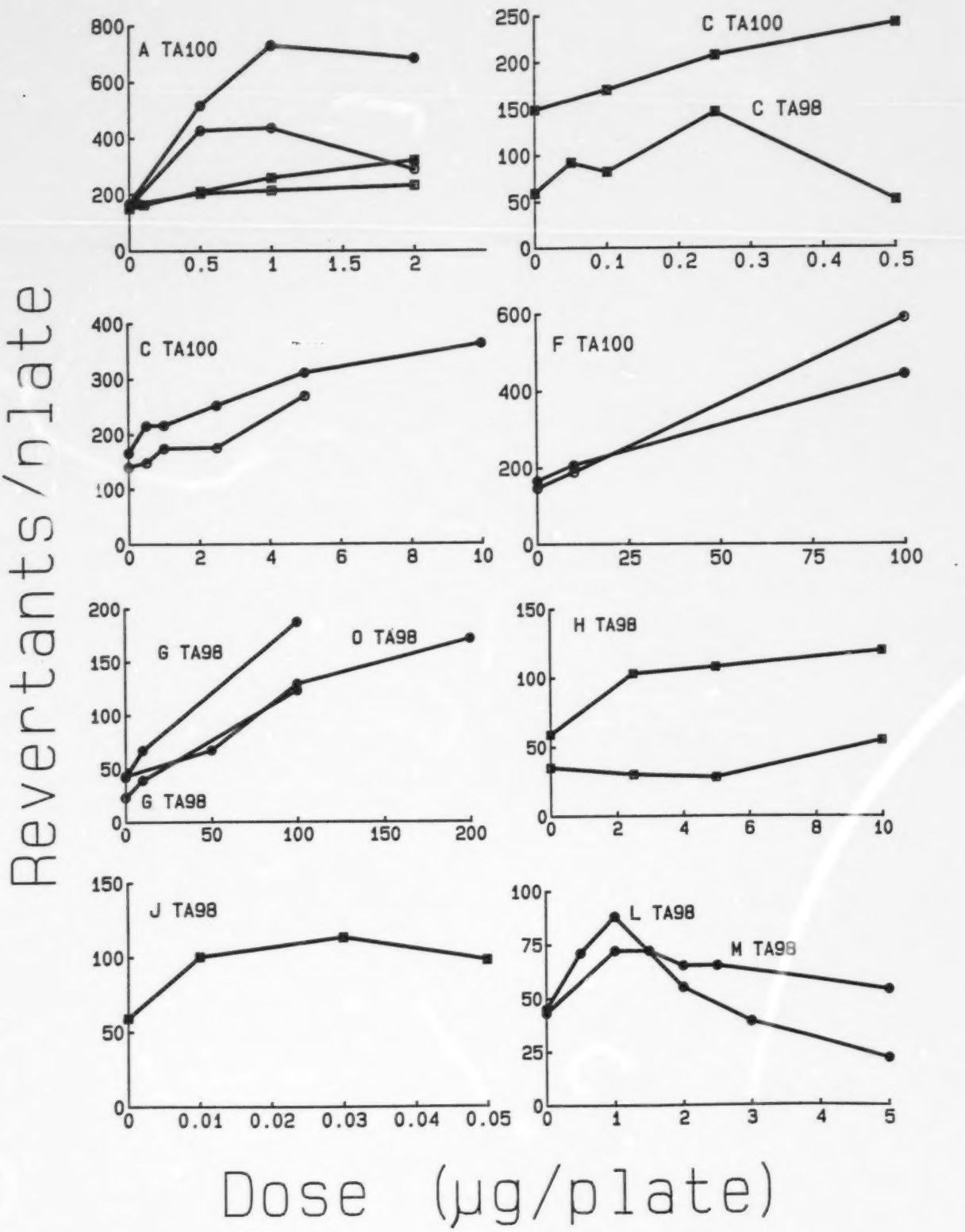
[b]NT, not tested.

LEGENDS TO FIGURES

Fig. 1. Dose-response curves of crude wastes (circles) and waste extracts (squares) in the presence (open markers) or absence (solid markers) of Aroclor 1254-induced rat liver S9. Experiments were performed as described in the Materials and Methods. Results are from representative experiments.

Fig. 2. Dose-response curves from strain TA98 of raw urines or urine extracts from F-344 rats administered crude wastes. Dosing of animals, preparation of urines and extracts, and bioassay protocols are described in Materials and Methods. Waste C urines were collected after 10 days of dosing; waste L and M urines were collected after a single dose performed as described in Materials and Methods. Results are from representative experiments.


Revertants/plate
Urine/C
0.2 g/kg
0.1 g/kg
0.05 g/kg
0.025 g/kg
0 g/kg
Urine/C
0.2 g/kg
C-18/urine
Raw Urine
Urine/L
2.5 g/kg
C-18/urine
Raw Urine
Urine/M
5 g/kg
C-18/urine
Raw Urine
Dose (ml or ml equivalents/plate)


A TA100
C TA100
C TA98
C TA100
F TA100
G TA98
O TA98
G TA98
H TA98
J TA98
L TA98
M TA98
Revertants/plate
Dose (µg/plate)